

REMARKS

The amendments to Claims 1, 4, 6 and 7 are pending.

Support for the amendments is found on page 6, 2nd paragraph; page 24, last two paragraphs, and FIG. 1.

No new matter has been entered by the amendments.

The rejection of Claims 14, and 6-7 under 35 USC 103(a) citing Soards et al and Roossinck et al is not applicable to the claims as the Soards construct deletes the entire 2b protein ORF whereas the claimed vector deletes the portion between the StuI site and the stop codon but retains the region from the initiation codon to the StuI site.

In the Office Action, the Examiner has indicated that "Soards et al. teach a cucumber mosaic virus Fny stain (CMV—Fny) viral vector wherein the 2b is deleted including the region from StuI to stop codon of the 2b ORF of pCY2 of instant application" (See lines 5—7, page 3 of the Office Action). Soards et al. is a CMV-Fny strain (Fny-CMVΔ2b) in which a sequence encoding 2b protein encoded in 2419-2713 of CMV RNA2, which is the entire nucleotide sequence encoding 2b protein, has been deleted. As is stated in lines 13 to 15 of col. 1 on page 648 of Soards: "Fny-CMVΔ2b only exhibits accumulation of a low level of CP (coat protein) at all times", and in the heading in lines 3 to 5 of the col. 2 on that same page: "Fny-CMVΔ2b exhibits slower progression than the wild strain", the efficiency of both infectivity and gene expression following transformation decreases. Soards et al. also states on page 649 that CMVΔ2b-GFP only exhibits limited spreading (line 11 of the left column and lines 8 and 9 of the Discussion of col. 2), and that deletion of 2bORF can effect virus replication (lines 17 and 18 of the Discussion of the col. 2).

Roossink et al. is cited for indicating that CMV-Y and CMV-Fny are related species. It does not, however, remedy the fact that Soard's fully deleted the 2b protein whereas the

present claims retain a part (Cf. Claim 1). Accordingly, the claims cannot be considered obvious as the art does not teach or suggest all of the claimed limitations.

Furthermore, the combined teachings of the references, and in particular Soards et al., merely indicates that virus infectivity and propagation decrease when 2b protein is deleted. Neither, however, teaches how to prepare plant virus vector using CMV-Y that is useful for plant transformation.

In addition, Applicants attach a publication by Matsuo et al (Planta (2007):225:277-286) who observed that the H1 vector that lacks the entire 2b ORF of CMV-Y can infect some but not all species, particularly, soybeans (see FIG. 4 and 5). In another publication by Chen et al (EMBO Rep. 9, 754 (2008)), it was observed that protein 2b is involved in RNA-silencing suppression (RSS) and it is a region between the initiation codon and StuI that shows the RSS. This, of course, means that the Soards et al vector lacking this region would not have RSS activity, which is very important for viral systemic movement.

Matsuo, Matsumura et al. teaches that they developed pCY2 as a viral vector by replacing the 2b gene (nucleotide positions 2420—2752) with a DNA fragment containing the cloning sites, generating a viral vector, C2—H1.

In this viral vector C2-H1, all of the 2b is deleted as shown in Fig. 2 of Matsuo. This viral vector C2—H1 corresponds to the viral vector disclosed in Soards wherein the 2b is deleted including the region from StuI site to stop codon of the 2b ORF of pCY2 of the present application.

The cDNA of aFGF, acidic fibroblast growth factor, was obtained by conventional PCR from human cDNA library. The aFGF clone was inserted between StuI and SpeI of the cloning sites in C2—H1 to obtain H1—aFGF as shown in Fig. 2.

Fig. 2 shows construction of the CMV—based vector and cloning of the aFGF gene. Cloning sites StuI, MluI and SpeI were introduced between nucleotide positions 2420 and

2752 in the CMV-Y RNA clone after deletion of 2b gene. Gene aFGF was cloned between the StuI and SpeI sites.

Fig. 4 of Matsuo shows expression of aFGF in plants by the viral vector C2-H1. Fig. 4a shows a Western blot of aFGF from *N. benthamiana* infected with H1-aFGF.

Lanes: P, aFGF produced by *E.coli* (25 ng, positive control); 1, inoculated leaf (200ng protein) 2, upper leaf (200ng protein) ; 3, healthy plant (200 ng protein) ; 4, leaf inoculated with H1 (vector). The numbers on the left indicate the positions of protein size markers.

Fig. 4b shows yield estimation of in planta-produced aFGF. Total soluble protein was isolated from the leaves infected with H1—aFGF. The original concentration of the protein extracts was 2.2 mg/ml. The 2-fold dilution series of total soluble proteins were separated by 8% SDS-PAGE, blotted onto PVDF membrane and probed with anti-aFGF antibody.

Lanes: P. *E. coli*-produced control aFGF (25ng); 1, contains 2.2 pg of total soluble protein (from the original extracts); 2-5, 2 fold dilution series of total soluble protein.

Based on the band density, it is estimated that the yield of aFGF is 5-8% of the total soluble protein.

In contrast, Fig. 5 shows a Western-blot analysis of aFGF from *G. max* and *A. thaliana*. Fig. 5a shows a Western—blot of aFGF from *G. max* plants infected with H1-aFGF. Lanes: P, aFOF produced by *E. coli* (25ng, positive control); 1, inoculated leaf (200 ng protein); 2, upper non-inoculated leaf (200 ng protein); 3, healthy plant (200 ng protein). The concentration of the original protein extracts for lane 1 was about 2 mg/ml.

Based on the 2-fold dilution series of control aFGF (right picture), the yield of aFGF from the soybean tissue was about 2.5% (about 5ng in lane 1).

Fig. 5b shows a Western-blot analysis of aFGF from *A. thaliana* plants infected with H1-aFGF. Lanes: P, aFGF produced by *E. coli* (25ng, positive control); 1, inoculated leaf; 2,

stalk of inoculated plant; 3, healthy plant. The concentration of the original protein extracts for lane 1 was about 0.7 mg/ml.

Based on the 2-fold dilution series of control aFGF from Arabidopsis tissues was about 1.5% (about 3ng in lane 1).

From these results, it is revealed that C2-H1 viral vector which lacks the 2b including the region from StuI site to the stop codon of the 2bORF of pCY2 may be used as a viral vector for *N. benthamiana* plants, but can not be satisfactorily used as a viral vector for *C. max* and *A. thaliana*.

The inventors have found that the CMV-Y viral vector wherein a region from an initiation codon to the StuI site of the ORF encoding 2b protein of RNA2 is retained and a region from a StuI site to a stop codon of an ORF encoding 2b protein of RNA2 is deleted is useful in order to solve the problems as mentioned above.

The CMV-Y viral vector of the present invention can be used as a viral vector for many kinds of plants including *N. benthamiana*, Soybean, Arabidopsis, etc.

Soards does not teach what Applicants have discovered and does not make the presently claimed vector obvious, even in view of the teachings of Roossinck.

The CMV-Y viral vector of the present invention is different from the viral vector disclosed in Soards in respect of the extent of the kinds of Plants to be applied and the extent of the efficient viral movement in such plants. In particular, the CMV-Y vector defined in the claims is a plant virus vector that demonstrates the remarkable and particularly preferable effect of not losing virus systemic infectivity, results in slower progression of symptoms than wild-type CMV-Y and allows an exogenous gene to be stably expressed, also in soybean plants

Applicants previously submitted a publication of Otagaki et al. (Plant Biotechnology, 2006, Vol. 23, pp. 259-265) as a reference indicating these advantages of the CMV-Y vector as claimed.

As described in the Otagaki publication, the CMV-Y vector (CMV2-A1 in Figure 1) only exhibits mild symptoms even when infected into *N. benthamiana* (lines 31 to 33 of the col. 2 on page 254), and has the characteristic of spreading throughout the entire plant, and this is also described in the specification (e.g., see page 8, 1st paragraph, page 9, 2nd paragraph, and the "Results" of the Example on page 28 in the specification).

There is nothing provided by the combination of cited art that CMV-Y, in which the 2bORF of RNA2 from StuI to the stop codon of the 2bORF has been partially deleted, while retaining the portion from the initiation codon to the StuI site demonstrates ideal properties as a vector. This must be so as Soards describes that CMV in which virus infectivity and spread decreased as a result of completely deleting the 2bORF of CMV-Fny.

Withdrawal of the rejection is requested.

A Notice of Allowance is requested.

Respectfully submitted,

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